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Non-alcoholic fatty liver disease caused by type IX glycogenosis – a series of cases in children

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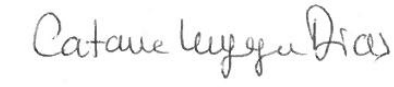

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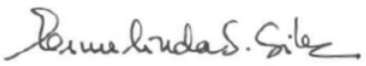

Assinatura do Orientador

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It is the author’s intention to submit this thesis for publication.

EPIGRAPH

“Eles não sabem, nem sonham,
Que o sonho comanda a vida,
Que sempre que um homem sonha
O mundo pula e avança
Como bola colorida
Entre as mãos de uma criança”

António Gedeão

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RESUMO

Introdução: A obesidade é, atualmente, uma pandemia à escala mundial, afetando crianças e adolescentes, produzindo múltiplas comorbilidades, uma das quais o Fígado Gordo Não-Alcoólico (NAFLD), cuja prevalência tem aumentado enormemente nos últimos anos. Na verdade, este aumento tem até ocorrido de forma mais acentuada do que o da obesidade e o consumo de frutose aparenta ser o fator de risco mais importante. Existem várias causas secundárias de esteatose hepática, mas devido à grande prevalência de fígado gordo associado à obesidade ou tipo de dieta, o diagnóstico de outras entidades clínicas torna-se difícil. Este diagnóstico torna-se especialmente importante quando se tratam de patologias com tratamento específico, como é o caso da Glicogenose tipo IX (GSD-IX).

Objetivos: Contribuir para o conhecimento dos diagnósticos diferenciais de NAFLD em idade pediátrica. Contribuir para a caracterização clínica, bioquímica, molecular e histológica, da GSD-IX, uma doença metabólica rara.

Metodologia: Estudo retrospectivo, a partir dos registos clínicos, de uma pequena série de casos (n=3) de GSD-IX, diagnosticados nos últimos 6 anos, atualmente em seguimento nas Unidades de Gastrenterologia ou de Doenças Metabólicas do Serviço de Pediatria do nosso hospital, e cuja forma de apresentação clínica foi NAFLD em idade pediátrica.

Resultados: Três doentes de sexo masculino, com apresentação de NAFLD antes dos 2 anos de idade, dois com diagnóstico confirmado antes dos 3 anos e um cujo diagnóstico só foi confirmado aos 11 anos. Nenhum dos doentes era obeso ou tinha excesso de peso; o consumo diário de frutose é desconhecido. A evolução foi favorável nos três doentes, com um tempo de seguimento que variou entre 2 e 6 anos.

Conclusões: Decidir até onde deve prosseguir a investigação de causas secundárias de NAFLD pode ser muito difícil. A GSD-IX deve fazer parte da lista de causas possíveis.

Palavras-chave: Glicogenose tipo IX; Fígado Gordo não Alcoólico; Esteatohepatite; Crianças

ABSTRACT

Background: Obesity is currently a worldwide pandemic, affecting children and adolescents, producing multiple co-morbidities, one of which is Non-Alcoholic Fatty Liver Disease (NAFLD), whose prevalence has increased dramatically in recent years. In fact, this increase has even occurred more markedly than with obesity, and fructose consumption appears to be the most important risk factor. There are several secondary causes of hepatic steatosis, but due to the high prevalence of fatty liver associated with obesity or type of diet, diagnosis of other clinical entities becomes difficult. This diagnosis becomes especially important when dealing with pathologies with specific treatment, such as glycogenosis type IX (GSD-IX).

Aims: Contribute to the knowledge of differential diagnosis of NAFLD in Pediatric age. Contribute to the clinical, biochemical, molecular and histological characterization of GSD-IX, a rare metabolic disorder.

Methods: A retrospective study of a small series of cases (n = 3) of GSD-IX diagnosed in the past 6 years, currently being followed up in the Units of Gastroenterology or Metabolic Diseases of the Pediatric Division of our hospital, and whose clinical presentation was NAFLD in Pediatric age.

Results: Three male patients with NAFLD before 2 years of age, two with confirmed diagnosis before age 3 and one whose diagnosis was confirmed at 11 years-old. None of the patients were obese or overweight; the daily consumption of fructose intake is unknown. The outcome was favourable in all of three patients, with follow-up period ranging from 2 to 6 years.

Conclusion: Deciding how far to investigate NAFLD secondary causes can be quite difficult, and GSD-IX should be on the list of possible causes.

Keywords: Glycogenosis type IX; Non-alcoholic fatty liver disease; steatohepatitis; children

LIST OF ABREVIATIONS

AH50 - Alternate Pathway Haemolytic assay
ALT – Alanine Aminotransferase
AST – Aspartate Aminotransferase
BMI – Body mass index
CH50 – Total haemolytic complement assay
CMV – Citomegalovirus
CPK – Creatine phosphokinase
EBV – Epstein-barr virus
GSDs – Glycogen storage diseases
GSD-III – Glycogenosis type III
GSD-VI – Glycogenosis type VI
GSD-IX – Glycogenosis type IX
LAL-D - Lysosomal acid lipase deficiency
NALFD – Non-alcoholic Fatty Liver Disease
NASH – Non-alcoholic Steatohepatitis
NASPGHAN - North American Society of Pediatric Gastroenterology, Hepatology and Nutrition
PhK – Phosphorylase kinase

INDEX

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The worldwide increase in the prevalence of childhood and juvenile obesity leads many patients to present with comorbidities in the Pediatric age [1, 2]. Among these, non-alcoholic fatty liver disease (NAFLD) is increasing at an even faster rate than obesity (incidence 34,2% in obese children; 7,6% in general population), and is already the most common cause of chronic liver disease in children and adolescents [3]. Unfavourable lifestyles, particularly a high-fructose diet, may be the cause [4]; Mediterranean diet appears to reduce the risk for non-alchoolic steatohepatitis (NASH) in obese Pediatric patients[5]. NAFLD may progress to fibrosis, culminating in the long term in cirrhosis and is projected as the primary indication for liver transplantation in the future [6].

NAFLD is characterized by fatty infiltration of the liver in the absence of alcohol or drug consumption, infections, malnutrition, and genetic/metabolic diseases. Therefore, diagnosis should only be assumed after excluding other causes, especially those with specific treatment [7]. Among the metabolic diseases to be considered are some glycogenosis [8].

Glycogenosis type IX (GSD-IX), one of the most common forms of glycogenosis (25% of cases; estimated frequency of 1/100 000), is caused by deficiency in hepatic phosphorylase kinase (PhK), which is composed by four subunits (α, β, γ, δ); GSD-IX subtypes are identified according to the affected subunit. Transmission can be linked to chromosome X (PHKA2 gene, 75% of all GSD-IX, subtype α,) or be autosomal recessive (gene PHKB, subtype β; gene PHKG2, subtype γ). Although the main substrate accumulated in the target organs is glycogen, there may be a predominance of lipids, particularly in the liver [9, 10].

We describe a small series of cases (n=3) of GSD-IX, subtype *α,* diagnosed and followed in the past 6 years, in the Pediatrics Division of our hospital, whose clinical presentation was NAFLD in Pediatric age.

CASE REPORTS

Case1

A male infant, first child of a healthy couple, was born after a 40-week pregnancy, with weight 3080g (p10-50) and length 48,5 cm (p10). At 18 months-old, he was hospitalized for pneumonia, presenting with hepatosplenomegaly and elevated transaminases [Aspartate Aminotransferase (AST) 67 UI/L, Alanine Aminotransferase (ALT) 53 UI/L]. Later-on, AST and ALT normalized but persistent hepatosplenomegaly lead to admission at our outpatient clinic by 25 months-old. At this moment, complementary investigation including lipidogramme, alpha-1-antitrypsin, creatine phosphokinase (CPK), ceruloplasmin, Hepatitis C virus antibodies was normal. Throughout the following years, spleen dimensions returned to normal, but the liver remained slightly enlarged with hyper-echogenicity. At 5 years-old, enzymatic activity in leucocytes for glycogenosis type III (GSD-III) was normal. He was discharged to his attending physician, and recommended to perform yearly abdominal ultrasounds. By 11 years-old, abdominal ultrasound revealed diffuse liver hyperechogenicity, suggesting steatosis. Laboratory tests performed are displayed in table 1. Liver histology revealed macro and micro-steatosis without inflammatory infiltrates or fibrosis (Fig.1). Copper in liver tissue was normal. Molecular study confirmed the previously described mutation c.1054C>T (p. 352X) exon 11 in hemizygosity on the PHKA2 gene. Dietary measures were prescribed. Currently, he's 17 years-old: transaminases remain normal; liver maintains steatosis pattern, with no adenomas; spleen is in the upper limit of normal size.

Case 2

A male infant, second child of a healthy couple, was born after a 37-week pregnancy with weight 2750g (p10-50) and length 46,5 cm (p10-50). He developed physiological jaundice that resolved within a week without phototherapy. During his first months, he had several episodes of wheezing, treated with Montelukast and fluticasone in aerosol. At 11 months of age, during a wheezing episode, transaminases (AST 158 UI/L; ALT 155 UI/L) and triglycerides (180 mg/dl) were elevated. At 15 months, he was admitted to our hospital for hepatomegaly (6cm below the costal grid, smooth surface, thin edge) and persistently elevated transaminases; abdominal ultrasound showed an enlarged and hyperechogenic liver with steatosis pattern; spleen was normal-sized. From laboratory tests we highlight the slightly low fasting glycemia and bicarbonate, discretely raised triglycerides and normal serum uric acid (table 1). Cardiac evaluation was normal. Molecular study showed a previously described causal mutation on the PHKA2 gene [c.892C>T (p. R298X - exon

9)] with hemizygosity pattern, confirming GSD-IXa. Dietary measures were prescribed. Two years later, hepatomegaly remained equal, although transaminases normalized; bicarbonates were slightly low and uric acid elevated. Patient emigrated to France and was lost from follow-up since.

Case 3

A male infant, first child of a healthy couple, was born after a 39-week high-risk pregnancy due to placental displacement, with weight 3300g (p10-50) and length 51cm (p50). In the first week, he developed jaundice (total bilirubin 17,3 mg/dl) treated with phototherapy. During the first 8 months he had multiple infections: *Escherchia coli* urinary tract infections, acute gastroenteritis, fever of undetermined origin, *Neisseria meningitidis* sepsis, *Coronovirus* upper respiratory infection, *Proteus* cystitis, acute bronchiolitis followed by acute otitis media and an episode of bacteriemia of unknown origin. This overwhelming amount of infections led to an investigation for complement deficiency, which showed reduced levels of total haemolytic complement assay (CH50) and alternate Pathway Haemolytic assay (AH50) and slightly increased C3 (174mg/dl) and C4 (48mg/dl), but no other immunological abnormalities. Throughout all these infectious episodes, patient had palpable hepatomegaly and consistently elevated transaminases. At 18 months, abdominal ultrasound showed an enlarged liver with steatosis pattern and a normal-sized spleen. Laboratory tests performed are displayed in table 1. A year later, molecular study confirmed the new and unrecognized mutation c.706G>T (p. E236*) exon 7 in hemizygosity in the PhKA2 gene, which leads to the production of a truncated protein. His mother is a carrier in heterozygosity. Diet measures were prescribed. Currently, he's 5 years-old and doing very well, with slight hepatomegaly (2 cm below costal margin), normal ALT (Fig.2) and steatosis pattern on ultrasound.

DISCUSSION

NAFLD is one of the comorbidities of obesity and its incidence is increasing dramatically. In addition, NAFLD has been associated with a certain dietary pattern, even without obesity or overweightness. Nonetheless, it can also be secondary to a large number of other entities [(7)]. Thus, a child with NAFLD, whether obese/overweight or not, becomes a challenge in terms of differential diagnosis.

The first struggle is to be certain of the presence of liver steatosis or steatohepatitis by non-invasive methods. ALT is currently the best screening method for fatty liver in children >10 years-old, with 88% sensitivity and 26% specificity for values >2 times the normal value[(11)]. Liver ultrasound may show a suggestive pattern but has low sensitivity. Liver histology, despite being the gold standard, is too invasive for a screening method [(12)].

None of our three patients were obese or overweight. All were symptomatic very early in life, with hepatomegaly and elevated transaminases, and liver ultrasound showing a pattern suggestive of steatosis. Only patient 1 had fatty liver confirmed through liver histology at 11 years-old, when raised level of 24h urinary copper obliged to disclose Wilson's disease [(13)].

The second struggle is the differential diagnosis approach for fatty liver. The 2017 North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) guidelines for diagnosis and treatment of NAFLD [(12)] don't provide enough orientation for a cost-benefit approach, regarding who and when should be screened for each listed genetic/metabolic disease. Furthermore, GSD-IX isn't even mentioned as a secondary cause of fatty liver.

In this case-series we took into account the age of the patients and prioritized the exclusion of diseases with specific treatment. Patient 1, at 11 years-old had been GSD-III excluded. Besides GSD types VI and IX and lysosomal acid lipase deficiency (LAL-D) [(14)], we also searched for Wilson's disease and juvenile haemochromatosis. In patient 2, the hypothesis of drug hepatotoxicity for Montelukast was considered, which could explain raised transaminases, but probably not hepatomegaly and steatosis. In patient 3, we considered the hypothesis of congenital immunodeficiency with liver injury, which wasn't confirmed. For the last 2 patients, LAL-D was also a possible cause, but was only tested on patient 3, since patient 2 was lost from follow-up.

Diagnosis of GSD-IXa was confirmed by molecular study in all patients. All had X-linked transmitted pathogenic mutations in the PHKA2 gene. In case 2 a new mutation was found, that leads to a premature stop codon, being accepted as a causal mutation.

GSD-IX clinical presentation is generally milder compared to other GSDs, (and very similar to GSD-VI), and symptoms (hepatomegaly, growth retardation, elevated transaminases, hypertriglyceridemia and sometimes ketosis and hypotonia) typically improve with age, being asymptomatic in adulthood[15]. However, genetic variants in PHKA2 have a broad phenotypic spectrum, including progression to cirrhosis [16, 17] and development of hepatic adenomas[18], as well as less common phenotypes with kidney dysfunctions due to renal tubular acidosis and central nervous system involvement with delayed cognitive and speech abilities [19]. So far, our patients' clinical course has been very benign.

Treatment consists in dietary measures, such as avoiding prolonged fastings and, when needed, drinking infusion with maltodextrin. More rarely, hyperuricemia or metabolic acidosis must be addressed. Untreated children may have undesirable repercussions such as morning sickness, which affects school performance, and growth retardation, causing psychological distress [20].

CONCLUSION

In summary, deciding when and how to search for secondary causes of NAFLD can be extremely difficult. All contributions to establish guidelines, possibly even an algorithm, are extremely important, making this task easier and more cost-effective. Also, GSD-IX should be included in these guidelines as a possible cause, as it can cause a spectrum of lesions from steatosis and steatohepatitis to cirrhosis.

APPENDIX

Table I - Baseline clinical and analytical features of patients at admission in reference centre

Clinical features	Case 1	Case 2	Case 3	Reference values
Sex	M	M	M	
Age (year of birth)	11 years	15 months	18 months	
Parental consanguinity	No	No	No	
Family history	negative	negative	negative	
Weight (kg) / percentil	43,6 / p50-85	11,0 / p50-85	11,0 / p50	
Length or Height (cm) / percentil	150,5 / p85	80 / p50-85	82 / p50	
BMI (kg/m^2) / percentile	19,25 / p50-85	17,2 / p50-85	16,4 / p50-85	
Hepatomegaly	No	Yes (5 cm)	Yes (3,5 cm)	
Splenomegaly	No	No	No	
Biochemical parameters				
Total bilirubin (mg/dl)	0,28	0,16	0,26	0,20 – 1,00
Conjugated bilirubin (mg/dl)	0,10	0,06	0,10	0,00 – 0,20
AST (UI/L)	26	**150**	**158**	10 – 34
ALT (UI/L)	16	**219**	**60**	10 – 44
γGT (UI/L)	13	35	24	10 – 66
CPK (UI/L)	136	95	NA	24 - 204
Glucose (mg/dl)	77	**66**	74	70-105
Bicarbonates (mmol/l)	**17,5**	**17,9**	**15,4**	22,0 – 29,0
Lactate	NA	1,28	1,63	0,5 – 2,20
Uric acid	4,7	3,4	4,0	2,0 – 5,5
Total cholesterol (mg/dl)	174	174	196	0 – 200
LDL-cholesterol (mg/dl)	94	116	136	0 – 130
HDL-cholesterol (mg/dl)	**70**	13	25	35 – 55
VLDL-cholesterol (mg/dl)	10	45	35	3 – 56
Triglycerides (mg/dl) 50	50	**227**	**174**	40 – 160

Other exams performed after				
Hepatitis A, B, and C CMV EBV	Negatives IgG+ IgM- IgG+ IgM-	Negatives IgG- IgM- IgG- IgM-	Negatives IgG+ IgM- IgG+ IgM-	
Serum alfa-1-antitrypsin (mg/dl)	Normal	Normal	normal	
Serum ferritin (ng/mL)	22	NA	NA	12,8-454
Transferrin saturation rate (%)	10	NA	NA	15-45
Serum ceruloplasmin (mg/dl)	26	NA	NA	16-36
Urinary copper 24h (μmol/d)	**1,086**	ND	ND	0,040-0,050
Copper in liver tissue (μg/g)	24,82	ND	ND	<40
Sweat-test	ND	Normal	ND	
LAL activity (in leucocytes)	Normal	ND	Normal	
Molecular studies				
PHKA2 gene	Mutation c.1054C>T (p. 352X) exon 11 in hemizygosity	Mutation c.892C>T (p. R298X) exon 9 in hemizygosity	Mutation c.706G>T (p. E236*) exon 7 in hemizygosity	

Legend: BMI – body mass index; CMV - Citomegalovirus; EBV - Epstein-barr virus; HDL – high-density lipoprotein; LAL –lysosomal acid lipase; LDL – low-density lipoprotein; NA – Non-Available; ND – Not-Done; VLDL – very low-density lipoprotein; γGT – gamma-glutamyl transferase

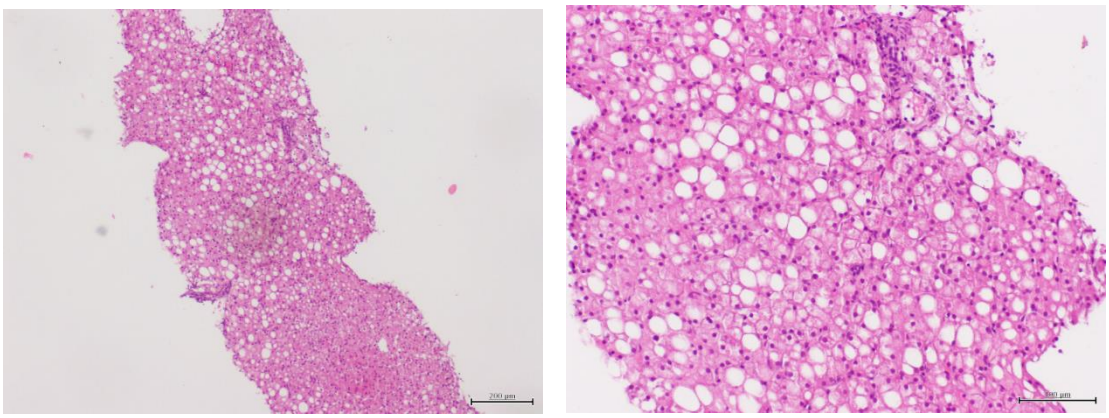

Fig.1 – Liver histology by 11 years-old in case 1, showing macro and micro-steatosis; hepatocytes with preserved morphology and dimensions within normal ranges, without inflammatory infiltrate or fibrosis.

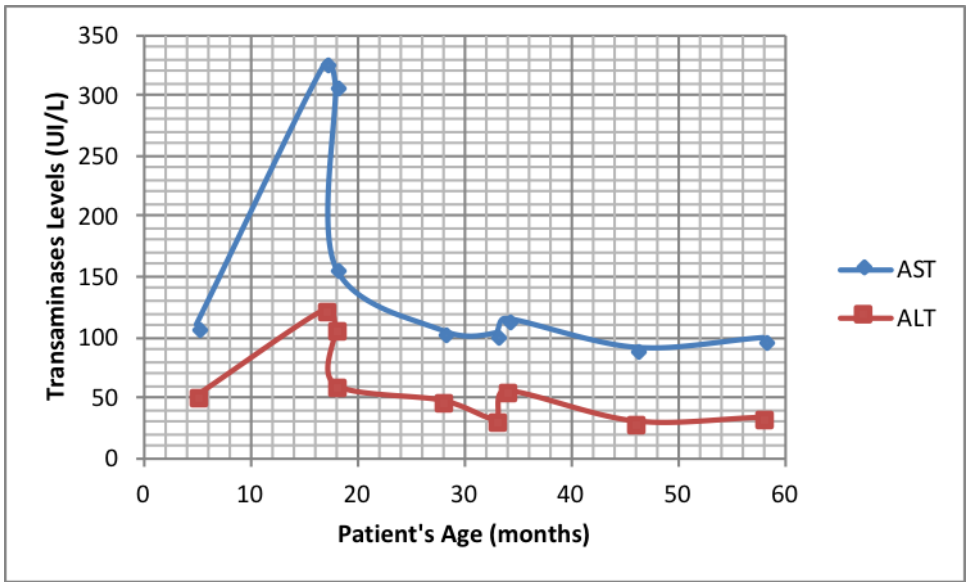

Fig.2 – Transaminases during follow-up in case 3

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